PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

(Chapter II of the Patent Cooperation Treaty)

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference BP110588/IR	**FOR FURTHER ACTION**	See Form PCT/IPEA/416
International application No. PCT/FI2004/000679	International filing date *(day/month/year)* 15.11.2004	Priority date *(day/month/year)* 14.11.2003
International Patent Classification (IPC) or national classification and IPC C07K14/465, C07K14/36, C12N15/62, C12N5/10		
Applicant NORDLUND, Henri Rainer et al.		

1. This report is the international preliminary examination report, established by this International Preliminary Examining Authority under Article 35 and transmitted to the applicant according to Article 36.
2. This REPORT consists of a total of 6 sheets, including this cover sheet.
3. This report is also accompanied by ANNEXES, comprising:
   a. ☒ *sent to the applicant and to the International Bureau)* a total of 3 sheets, as follows:
      ☒ sheets of the description, claims and/or drawings which have been amended and are the basis of this report and/or sheets containing rectifications authorized by this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions).
      ☐ sheets which supersede earlier sheets, but which this Authority considers contain an amendment that goes beyond the disclosure in the international application as filed, as indicated in item 4 of Box No. I and the Supplemental Box.
   b. ☐ *(sent to the International Bureau only)* a total of (indicate type and number of electronic carrier(s)) , containing a sequence listing and/or tables related thereto, in computer readable form only, as indicated in the Supplemental Box Relating to Sequence Listing (see Section 802 of the Administrative Instructions).

4. This report contains indications relating to the following items:

☒	Box No. I	Basis of the opinion
☐	Box No. II	Priority
☐	Box No. III	Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
☒	Box No. IV	Lack of unity of invention
☒	Box No. V	Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
☒	Box No. VI	Certain documents cited
☐	Box No. VII	Certain defects in the international application
☐	Box No. VIII	Certain observations on the international application

Date of submission of the demand	Date of completion of this report
14.09.2005	01.03.2006
Name and mailing address of the international preliminary examining authority: European Patent Office - P.B. 5818 Patentlaan 2 NL-2280 HV Rijswijk - Pays Bas Tel. +31 70 340 - 2040 Tx: 31 651 epo nl Fax: +31 70 340 - 3016	Authorized Officer Kools, P Telephone No. +31 70 340-1964

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

International application No.
PCT/FI2004/000679

Box No. I Basis of the report

1. With regard to the **language**, this report is based on the international application in the language in which it was filed, unless otherwise indicated under this item.

   ☐ This report is based on translations from the original language into the following language , which is the language of a translation furnished for the purposes of:
   - ☐ international search (under Rules 12.3 and 23.1(b))
   - ☐ publication of the international application (under Rule 12.4)
   - ☐ international preliminary examination (under Rules 55.2 and/or 55.3)

2. With regard to the **elements*** of the international application, this report is based on *(replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report)*:

   Description, Pages

   1-40 as originally filed

   Claims, Numbers

   1-21 filed with telefax on 14.12.2005

   Drawings, Sheets

   1/18-18/18 as originally filed

   ☒ a sequence listing and/or any related table(s) - see Supplemental Box Relating to Sequence Listing

3. ☐ The amendments have resulted in the cancellation of:
   - ☐ the description, pages
   - ☐ the claims, Nos.
   - ☐ the drawings, sheets/figs
   - ☐ the sequence listing *(specify)*:
   - ☐ any table(s) related to sequence listing *(specify)*:

4. ☐ This report has been established as if (some of) the amendments annexed to this report and listed below had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2(c)).
   - ☐ the description, pages
   - ☐ the claims, Nos.
   - ☐ the drawings, sheets/figs
   - ☐ the sequence listing *(specify)*:
   - ☐ any table(s) related to sequence listing *(specify)*:

* *If item 4 applies, some or all of these sheets may be marked "superseded."*

Box No. IV Lack of unity of invention

1. ☐ In response to the invitation to restrict or pay additional fees, the applicant has:
   - ☐ restricted the claims.
   - ☐ paid additional fees.
   - ☐ paid additional fees under protest.
   - ☐ neither restricted nor paid additional fees.

2. ☒ This Authority found that the requirement of unity of invention is not complied with and chose, according to Rule 68.1, not to invite the applicant to restrict or pay additional fees.

3. This Authority considers that the requirement of unity of invention in accordance with Rules 13.1, 13.2 and 13.3 is

   ☐ complied with.

   ☐ not complied with for the following reasons:

4. Consequently, this report has been established in respect of the following parts of the international application:

   ☒ all parts.

   ☐ the parts relating to claims Nos. .

Box No. V Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	1-21
	No:	Claims	
Inventive step (IS)	Yes:	Claims	1-21
	No:	Claims	
Industrial applicability (IA)	Yes:	Claims	1-21
	No:	Claims	

2. Citations and explanations (Rule 70.7):

   see separate sheet

Box No. VI Certain documents cited

1. Certain published documents (Rule 70.10)

   and / or

2. Non-written disclosures (Rule 70.9)

   see separate sheet

Supplemental Box relating to Sequence Listing

Continuation of Box I, item 2:

1. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application and necessary to the claimed invention, this report has been established on the basis of:

   a. type of material:

   ☒ a sequence listing

   ☐ table(s) related to the sequence listing

   b. format of material:

   ☒ in written format

   ☒ in computer readable form

   c. time of filing/furnishing:

   ☒ contained in the international application as filed

   ☒ filed together with the international application in computer readable form

   ☐ furnished subsequently to this Authority for the purposes of search and/or examination

   ☐ received by this Authority as an amendment on

2. ☐ In addition, in the case that more than one version or copy of a sequence listing and/or table(s) relating thereto has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional observations, if necessary:

Re Item I

Basis of the report

The opinion is based on the amended claims 1-21 which appear to meet the requirements of Article 19(2) PCT.

Re Item IV

Unity of invention

The present set of claims is unified, due to the restriction to the dual-chain Avidins.

Re Item V

Reasoned statement with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Reference is made to the following document:

D1: US-B1-6 492 492 (STAYTON PATRICK S) 10 December 2002 (2002-12-10)

2. Novelty

2.1 The present application meet the criteria of Article 33(1) PCT, because the subject-matter of claim 1 is new in the sense of Article 33(2) PCT.
Document D1 discloses circular permuted (strept)avidin monomers having the N and C terminus fused and the circular chain reopened at a different position in the chain. One disclosed mutant can be interpreted as having the new N terminus before beta6 and the C-terminus after beta5 (see Figure 1, top). It further discloses 1-6 amino acid peptide linker sequences comprising Glycine and Serine residues. The resulting mutants have an altered biotin binding affinity. However, dual chain avidins are not disclosed.

2.2 The subject-matter of claims 2-21, i.e. pseudo-tetrameric avidin based on said dual chain avidins and single chain avidins comprising two fused dual chain avidins, their encoding polynucleotides, recombinant vectors comprising said polynucleotides, recombinant host cells comprising said vectors and methods of producing said polypeptides is also new.

3. Inventive step

3.1 The closest prior art to the subject-matter of claim 1 is D1 (for details on its disclosure see point 2.1 above). The subject-matter of claim 1 differs from this known avidin mutants in that: two units of avidin are interconnected so that they reside on one protein chain.

3.5 The problem to be solved by the present invention may therefore be regarded as the provision of mutant avidin proteins having a higher number of biotin binding sites.

3.6 The solution proposed in claim 1 of the present application is considered as involving an inventive step (Article 33(3) PCT) for the following reasons. Neither the prior art D1, nor other prior art, contains any incentive to change a native avidin protein in a protein consisting of two mutated avidin units. There is also no indication that two mutated avidin molecules in one polypeptide chain would form a functional biotin binding protein with more biotin binding sites. Therefore, the skilled person has no means to arrive at the presently claimed subject-matter without performing an inventive step.

3.7 A similar reasoning applies, mutatis mutandis, to the subject-matter of claims 2-21.

4. Industrial applicability

4.1 The subject-matter of claims 1-21, have industrial applicability. The avidin mutants, especially the double chain avidins and pseudo tetramers find there use in designing mutants forms with specific biotin binding affinity and other characteristics.

Re Item VI

Certain documents cited

The ISR cites one PX document. This document has no effect on the novelty of the presently claimed subject-matter as the priority appears to be valid.

Claims

1. A dual-chain avidin (dcAvd), **characterized** in that it comprises a fusion of two of the monomers selected from the circularly permuted monomers of circularly permuted avidin wherein the new N-terminus is before β-strand 5 and the new C-terminus after β-strand 4 (cpAvd5→4), circularly permuted avidin wherein the new N-terminus is before β-strand 6 and the new C-terminus after β-strand 5 (cpAvd6→5), and circularly permuted avidin wherein the new N-terminus is before β-strand 4 and the new C-terminus after β-strand 3 (cpAvd4→3), where the carboxyl terminal amino acid and the amino terminal amino acid of the polypeptide of an avidin monomer have been joined directly or via a linker, and new carboxyl and amino termini have been created to the polypeptide, and the resulting circularly permuted avidin monomer binds biotin or other ligand,

2. The dual-chain avidin of claim 1, **characterized** in that the avidin is selected from wild type avidin, mutant form of avidin, streptavidin and variant of avidin, such as other poultry avidins and chicken avidin-related proteins (AVRs).

3. The dual-chain avidin of claim 1, **characterized** in that the carboxyl terminal amino acid and amino terminal amino acid have been joined by a linker comprising one or more amino acids.

4. The dual-chain avidin of claim 3, **characterized** in that the linker is a hexapeptide comprising four glycine residues and two serine residues and wherein one glycine is connected to the carboxyl terminal amino acid and one serine is connected to the amino terminal amino acid.

5. The dual-chain avidin of claim 1, **characterized** in that the biotin-binding affinity of the circularly permuted avidin is different from the wild type avidin biotin-binding affinity.

6. The dual-chain avidin of claim 1, **characterized** in that the HABA-binding affinity of the circularly permuted avidin is different from the wild type avidin HABA-binding affinity.

7. The dual-chain avidin of claim 1, **characterized** in that the monomer has been mutated.

8. The dual-chain avidin of claim 7, **characterized** in that the monomer has been mutated by changing the tyrosine residue 33 to any other amino acid residue X and/or the isoleucine residue 117 to any other amino acid residue X and/or the serine residue 16 to any other amino acid residue X and/or the threonine residue 35 to any other amino acid residue X and/or the asparagine residue 118 to any other amino acid residue X, (Y33X, I117X, S16X, T35X, N118X).

9. The dual-chain avidin of claim 8, **characterized** in that the monomer has been mutated by changing the tyrosine residue 33 to histidine residue and/or the isoleucine residue 117 to cysteine residue and/or the serine residue 16 to alanine residue and/or the threonine residue 35 to alanine residue and/or the asparagine residue 118 to methionine, (Y33H, I117C, S16A, T35A, N118M).

10. A dual-chain avidin of claim 1, **characterized** in that the two monomers are fused together directly or joined by means of a spacer.

11. A dual-chain avidin of claim 10, **characterized** in that the spacer is a peptide spacer from about 1 to 40 amino acid residues.

12. A dual-chain avidin of claim 11, **characterized** in that the spacer is a peptide SGG or SGGS.

13. A dual-chain pseudo-tetrameric avidin, **characterized** in that it comprises two dual-chain avidins (dcAvd).

14. A dual-chain pseudo-tetrameric avidin of claim 13, **characterized** in that it binds biotin.

15. A single-chain avidin (scAvd), **characterized** in that it comprises two dual-chain avidin (dcAvd) molecules of claim 13 fused together to form a single polypeptide.

16. A single-chain avidin of claim 15, **characterized** in that the dcAvd-molecules are fused together via a linker.

17. A single-chain avidin of claim 16, **characterized** in that the linker is a 12 amino-acid linker GGSGSGSGSGSG.

18. An isolated polynucleotide encoding any of the avidin proteins of claims 1-17.

19. A recombinant vector comprising the polynucleotide of claim 18, wherein the polynucleotide is DNA.

20. A recombinant host cell comprising the polynucleotide of claim 18, wherein said polynucleotide is DNA.

21. A method for producing a polypeptide comprising expressing from the recombinant cell of claim 20 the polypeptide encoded by said polynucleotide.